


**Data Evaluation Record on the Acute Toxicity of Fluopyram (AE C656948) +
Tebuconazole (HWG 1608) SC 400 G to Freshwater Invertebrates- Water flea (*Daphnia magna*)**

EPA MRID Number 47567611

Data Requirement: EPA DP Barcode: D386298
EPA Guideline: OPPTS 850.1010

Test material: FLU+TBZ SC200+200A G **Purity:** 17.1% w/w FLU, 17.5% w/w TBZ
Common name
Chemical name: Fluopyram (AE C656948) and Tebuconazole (HWG 1608)

Primary Reviewer: Stephen Carey, Biologist
EPA/OCSPP/OPP/EFED/ERB6

Signature:  7/28/11
Date: 7/28/11

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Date:

Reference/Submission No.: {.....}

EPA PC Code 080302/128997

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Executive Summary:

In a 48-h static test system the acute toxicity of fluopyram & tebuconazole SC 400 (200+200) G (formulation containing active ingredients fluopyram with 17.1% w/w and tebuconazole with 17.8% w/w) to *Daphnia magna* (1st instars < 24 h old, 6 x 5 animals per concentration) was studied at nominal concentrations of 0, 6.25, 12.5, 25.0, 50.0 and 100 mg product/L. The concentrations of the product were not measured; results are based on nominal concentrations. Tebuconazole was not measured; however, fluopyram was measured for verification of the exposed test concentrations. The test conditions met all validity criteria, given by the mentioned guidelines. The accompanying chemical analysis of fluopyram in the freshly prepared test solutions at test initiation revealed concentrations between 103% and 108% (mean: 106%) of the corresponding nominal concentrations. The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 101% and 111% (mean: 108%) of nominal. No contaminations of fluopyram were detected in samples from untreated water control. In the controls, no immobility or sub-lethal findings were observed. In test level 50.0 mg/L behavioral changes such as lie at the bottom of tank and quick trembling antennae movements were observed during the entire exposure period.

After 24 and 48 h, no immobilization of daphnids was observed at concentrations up to 25 mg product/L. At 50 mg product/L, 3.3% and 30% immobilization were observed after 24 and 48 h, respectively. At 100 mg product/L, 100% immobilization was observed after 24 h. Based on nominal concentrations, the 48 h - EC₅₀ was calculated to be 56.90 mg product/L (C.I.95%: n.d.). The NOAEC for both immobility and sub-lethal effects was 25.0 mg product/L.

The toxicity study is scientifically sound but does not satisfy EPA guideline requirement for an acute freshwater invertebrate toxicity study with water flea. The study is classified as supplemental since the test concentrations of the product were not measured.

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I) Material and Methods

Guidelines Followed: OECD guideline 202,(2004); EEC Directive 92/69/EEG, part C.2 (1992); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72.2 (1982), OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).

Compliance: The study was conducted in compliance with, and satisfies the requirements of:

- OECD Principles of GLP (1982)
- Annex 1 ChemG Principles of GLP (2002)

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. Materials

- Test material:** Fluopyram (AE C656948) + tebuconazole (HWG 1608) SC 200 + 200

Specification No: 102000016375

Batch No: 2006-008040

Purity: Nominal: 200 g fluopyram/L + 200 g tebuconazole/L
Analyzed: 193.4 g fluopyram /L (17.1 % w/w) and 198.0 g tebuconazole /L (17.5% w/w)

Visual appearance: beige suspension

Density: 1.130 g/mL
- Test organism:** Water flea (*Daphnia magna*)

Age: 1st instars; <24 hrs old

Source: Bayer laboratory stock breeding cultures

Acclimation period: Maintained in 2000 mL containers (50 to 100 daphnids per container) in weekly renewed aqueous media (test medium), being placed in an climate-controlled environment under study conditions. They were fed three times per week with living cells of the green algae *Desmodesmus subspicatus*.
- Environmental conditions**

Water quality: Artificial water (type M7) is prepared for breeding of the stock culture and for testing as defined by the underlying OECD / EEC guidelines. Test water was reconstituted water prepared by dissolving mineral salts to demineralized water (conductivity <10 µS/cm). The prepared test water was aerated and tempered to 20 °C for at least 48h prior to start of the study. Immediately before use in a study, the vitamin components were added, using a separately deep frozen stored stock solution.

Temperature: 20.8 to 21.2 °C

Photoperiod: 16 hours light / 8 hours dark

pH: 8.1

O₂ content: 98 – 99 % of control (control with 8.9 and 8.5 mg/L at start and end of test respectively)

Total organic carbon: <2 mg/L

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Particulate matter: <1 mg/L
 Metals: <1 mg/L
 Pesticides: <0.05 mg/L
 Chlorine: <0.01 mg/L

B. Study design and methods

1. In life dates:	2007-03-27 to 2007-03-30
2. Experimental treatment and observations:	
Test duration:	48 hours
Test system:	Static
Test vessel material:	Glass
Test medium volume:	50 mL
Number of test levels:	1 water control, 5 treatment concentrations
Applied concentrations:	
Nominal:	Formulation: 0 (control), 6.25, 12.5, 25.0, 50.0 and 100 mg test item/L Fluopyram: 0 (control), 1.07, 2.14, 4.28, 8.56, 17.12 mg a.i./L <0.104 (LOQ, control), 1.14, 2.18, 4.62, 9.37 and 18.2 mg ai/L
Measured (mean):	
Number of replicates:	Six replicates of 5 daphnids per test level
Number of daphnids per test level	30
Solvent carrier:	None
Solvent load:	-
Reference substance:	K ₂ Cr ₂ O ₇
Test system:	Static
Test treatment level (nominal):	0 (control), 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L
Test result:	24 h – EC ₅₀ = 1.04 mg/L

Exposure

Neonates of the water flea *Daphnia magna* were exposed for 48 hours to fluopyram & tebuconazole SC 400 (200+200) g/L in aqueous suspension without adding any solvent or dispersant. Six test vessels with 50 mL test solution and five daphnids were utilized per treatment group and control (=30 animals per study group). The beakers were covered with transparent glass plates and placed in a climate controlled environment (isolated chamber) between 18 and 22 °C (maximum allowed deviation ± 1 °C within 48 hours). They were illuminated by "cool white" fluorescent bulbs in a 16:8 hours light dark cycle, at a light intensity of max. 1200 lux. The water fleas were not fed and the test solutions were not artificially aerated during exposure. The study covered five geometrically spaced nominal concentrations (6.25, 12.5, 25.0, 50.0 and 100 mg product/L = factor 2.0, supplemented by an untreated dilution water (blank) control. Preparation of test solutions occurred immediately before the start of exposure. Appropriate amounts of the test substance were directly added to the test water (Elendt M7) to establish the nominal test concentrations

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3. Observations:

Endpoints and water quality

After 24 and 48 hours, behavior of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel. Additionally, all possible signs of sublethal effects were recorded. Prior to preparation of the exposure solutions, conductivity, pH, total hardness and alkalinity of the dilution media (Elendt M7) were determined. The dissolved oxygen and pH values were measured in the freshly prepared test solutions of each treatment level and control and also in the pooled replicates of the aged media at test termination (day 2). Light intensity was measured at start of the study as "diffuse light" immediately above the exposure vessels with a photometer. Environmental temperature was continuously recorded during the test by a computer controlled measurement system. Additionally, temperature of the test media was measured inside one vessel of the untreated control and of the highest test concentration at start and end of the study.

Statistical methods

For EC₅₀ determination, a dose response relationship curve was modeled by Probit Analysis after Finney fitted by an iterative weighed linear regression according to the Maximum Likelihood principle, which allows computation of EC₅₀ and 95 percent confidence limits for immobility rates if possible (mathematical limits based on quality of the dose-response pattern). The described statistical procedures are carried out by using the ToxRat Professional Software, Vers.2.09 of the ToxRat Solutions GmbH, Germany.

Analytical verification

For verification of the aspired exposure concentrations, content of the active substance AE C656948 was analytically determined. The other active ingredient tebuconazole was not analyzed since it is present in the formulated product in a fixed ratio to the analyzed component. Analytical determinations of fluopyram were made by HPLC-UV. The LOQ is 5 µg/L and the LOD 1.7 µg/L. Fluopyram concentrations were analyzed in the test medium at the beginning (Day 0) and at test termination (48h).

I) Results and Discussion

Findings and observations

Analytical data:

The accompanying chemical analysis of AE C656948 in the freshly prepared test solutions at test initiation revealed concentrations between 103% and 108% (mean: 106%) of the corresponding nominal concentrations. The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 101% to 111% (mean: 108%) of nominal. No contaminations of AE C656948 were detected in samples from untreated water control.

Behavioral effects and mortality:

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No immobility's or other effects on behavior occurred in untreated control over 48 hours of exposure. Data on reported immobility and sub-lethal effects of daphnids (*D. magna*) are presented in **Tables 1 and 2**, respectively, below.

Table 1: Reported Immobility of Daphnids (*D. magna*) Exposed to Fluopyram + Tebuconazole SC 200 + 200.

Nominal concentration [mg product/L]	Mean measured concentration [mg fluopyram/L]	Immobilization after 48 hours	
		[n]	[%]
0 (control)	<0.104 (LOQ)	0	0
6.25	1.14	0	0
12.5	2.18	0	0
25.0	4.62	0	0
50.0	9.37	9	30
100.0	18.2	30	100
NOAEC (48 h): 25.0 mg product/L (nominal)			
EC ₅₀ (48 h): 56.90 mg product/L (nominal)			

Table 2: Reported Sub-lethal Effects of Daphnids (*D. magna*) Exposed to Fluopyram + Tebuconazole SC 200 + 200.

Nominal concentration [mg product/L]	Mean measured concentration [mg fluopyram/L]	Signs of toxicity after 48 hours	
		No. affected	Signs
0 (control)	<0.104 (LOQ)	0	0
6.25	1.14	0	0
12.5	2.18	0	0
25.0	4.62	0	0
50.0	9.37	6	1, 6
100.0	18.2	30	n/a – all dead
NOAEC (48 h): 25.0 mg product/L (nominal)			
EC ₅₀ (48 h): n.d.			

1 – quick antennae movements; 6 – lie at the bottom

II) Conclusion of study author:

The following EC₅₀ for immobilization of *Daphnia magna* after 24 and 48 hours of static exposure to AE C656948+Tebuconazole SC 200+200 in aqueous solution were found:

Probit Analysis for Data obtained after	EC ₅₀ in mg product/L (nominal)	Lower 95% CI in mg product/L (nominal)	Lower 95% CI in mg product/L (nominal)
24 hours	69.84	n.d.	n.d.
48 hours	56.90	n.d.	n.d.
n.d.= not determined due to mathematical reasons			

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EPA Reviewer's Conclusion:

The test concentrations of the formulation product were not measured. This affects the acceptability of the test.

The Reviewer agrees with the following endpoints from this acute toxicity study on water flea (*daphnia magna*) exposed to Fluopyram + Tebuconazole SC 400 G.

EC ₅₀ (based on immobility):	56.90 mg product/L nominal	95% C.I.: n.d.
NOAEC (based on both immobility and sub-lethal effects):	25.0 mg product/L nominal	

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